IMMUNOCHEMICAL QUANTITATIVE DETERMINATION OF SOME SERUM PROTEINS AND THEIR DIAGNOSTICAL IMPORTANCE

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In the past few years our knowledge of serum proteins has been largely extended thanks to the introduction of immunochemical methods. Barely after discovering their great significance, the immunochemical analysis of serum proteins made possible the determination and identification of more than 100 protein components in the blood serum (1, 5, 8). Immunoelectrophoretic determination of serum proteins failed to gain recognition, and accordingly it was never applied on a large scale. The attractive pictures disclosed by the stratification of proteins in the sera under study, and in the biologicalg luids of the organism hardly lend themselves to interpretation. Followine-the introduction of monospecific antisera with the purpose to identify the numv rous precipitation arcs of serum proteins, and to perform their quantitatite determination, the great power of immunochemical methods became eviden. Many authors no longer refer to several total protein fractions, but rather to strictly defined and comprehensively studied protein constituents in the blood serum (4, 6, 9, 11, 12).

Nowadays radial immunodiffusion in agar has gained popularity as the most feasible method, suggested in 1957 by F. Feinberg, and further improved relative to international standards by G. Mancini et al (1965) and by J. L. Fahey as well (cited by 4).

Since 1971, in the clinic of infectious diseases in Varna quantitative serum proteins' determination was undertaken, initially of serum immunoglobulins among patients with acute viral hepatitis, and later — in other infectious and noninfectious diseases. Along with that, other proteins such as transferrin, alpha$_2$-macroglobulin, complement C 3 (β_1C/β_1A-globulin), prealbumin, alpha$_1$-antitrypsin, beta$_2$-glycoprotein etc. were also determined. In this report the results of the listed above studies, performed over the period 1971 through 1976, and their diagnostical implication in the clinical practice are presented.

Material and Methods

Patients with viral and bacterial infectious enteral diseases, respiratory droplet viral diseases, chronic hepatic affections, malignant hemopathies, bronchial asthma etc. were investigated. They were distributed in the following groups: viral hepatitis (VH) comprising various clinical forms — 218, bacterial infections — 459 (dysentery, confirmed bacteriologically — 91, clinical dysentery — 132, salmonellosis — 40, alimentary toxicoinfections — 70, enterocolitis — 89, other intestinal diseases — 37), influenza — 81, epidemic

parotitis — 15, bronchial asthma — 50, chronic hepatic diseases — chronic hepatitis and cirrhosis — 177, blood diseases — 150. Determination of serum proteins was performed through radial immunodiffusion after G. Mancini (10). Immunodiffusion plaques obtained from Behringwerke (BRD) and Hyland (USA) were made use of. Regardless of the many advantages the method just referred to is expensive because of the high commercial cost of the immunodiffusion plaques, and the necessity to import them from capitalist countries. This led us to undertake the preparation of immunodiffusion plaques by ourselves over object glasses with agar, using monospecific antisera IgG and IgA, obtained from the Institute of Infectious and Parasitic Diseases — Sofia. The comparative study of the two methods of work showed that the results recovered were equivalent. No difference whatsoever was established between them. On the contrary, it was found that local monospecific antisera were in no way inferior to those offered and purchased from the various foreign firms. The mean values of serum proteins from 300 healthy individuals — blood donors from Varna — served as controls.

Results and Discussion

The results of quantitative serum proteins' determination show that serum immunoglobulins IgG, IgM, IgA and IgE display an increase of the type of

Table 1

Values of Some Serum Proteins in Viral Hepatitis and Epidemic Parotitis

Indicators / rcurs atients		No		Serum proteins						
				Immunoglobulins				Alpha$_2$ macroglobulin	Transferrin	C_3 1 C/ 1 A-gl
				Ig G	Ig M	Ig A	Ig E			
				mg 100 ml				mg 100 ml		
Viral hepatitis		138	$\bar{x}$	2295,95	118,85	324,08	356,77	307,31	268,44	112,87
			σ	755,40	71,77	110,37	107,70	97,22	36,12	36,53
			m_x	61,07	5,80	8,92	13,26	7,86	8,90	6,67
Australian antigen	(+)	63	$\bar{x}$	2530,91	88,41	320,00	313,52	274,55	324,77	—
			σ	600,47	51,55	100,12	85,35	86,35	86,74	
			m_x	104,14	7,77	15,20	13,08	12,87	11,42	
	(—)	75	$\bar{x}$	2331,11	124,72	375,67	308,19	244,58	251,39	—
			σ	603,96	76,99	126,57	98,91	81,26	73,72	
			m_x	100,66	12,83	21,20	16,49	13,54	12,29	
Epidemic parotitis		132	$\bar{x}$	1922,67	83,67	132,00	—	—	—	103,57
			σ	559,07	44,86	43,58				31,86
			m_x	114,46	11,59	11,26				6,85
Controls		28	$\bar{x}$	1200,11	80,02	288,22	124,00	250,44	280,00	89,21
			σ	319,00	29,14	121,01	31,01	96,67	55,53	18,41
			m_x	60,27	15,11	22,86	5,85	18,27	17,57	3,49

Table 2

Serum Immunoglobulin and Some Serum Protein Values in Viral Hepatitis and Bronchial Asthma

Disease			Immunoglobulins				Other proteins		
			Ig E	Ig G	Ig M	Ig A	α_2-macrogl.	Transferrin	
			U	mg%	mg%	mg%	mg %	mg %	
Viral hepatitis		80	311,13	2441,00	104,75	345,50	261,06	291,75	
			92,45	660,48	66,73	116,26	84,86	83,27	x
			10,34	73,88	7,46	13,00	9,49	9,31	
Australian antigen	(+)	44	313,52	2530,91	88,41	320,00	247,55	324,77	
			86,74	690,47	51,55	100,12	85,35	75,74	x
			13,08	104,14	7,77	15,10	12,87	11,42	
	(—)	34	308,19	2331,11	124,72	376,58	244,58	251,39	
			98,91	603,96	76,90	126,57	81,26	73,72	x
			16,49	100,66	12,83	21,10	13,54	12,29	
Bronchial asthma		50	394,15	2620,00	67,40	366,64	214,80	279,80	
			192,51	775,72	40,19	100,14	71,41	53,47	x
			27,23	109,72	5,68	14,16	10,10	7,56	
Controls		15	124	1200	80	238	250	280	
			31	319	29	121	60	67	x

polyclonal gammopathies, optimally expressed in VH. In the acute phase of the disease, a high concentration of the serum IgM level was observed, followed by an increase in IgG and IgA. This was already demonstrated in earlier studies of the authors (2, 3). IgE which is one of the rather recently discovered serum proteins with antibody qualities, was increased in a great percentage of VH patients — three times as much as in the controls. This points to the presence of an allergic component in VH pathogenesis. IgE was likewise substantially elevated in patients with bronchial asthma.

The serum level of transferrin proved to be increased. Low transferrin levels were established only in two VH cases with dystrophic form of the disease, complicated by hepatal coma and lethal outcome. Researches by Bulgarian authors in a limited number of patients with a past history of VH disclosed a reduction of transferrin (7). This is an indication that following VH affection, the function of the liver is impaired for a certain period of time with synthesis of transferrin being likewise disturbed, and its serum level — lowered in the posthepatitis stage. The increase in the other serum protein — alpha$_2$-macroglobulin — was rather pronounced among the cases with cholestatic component in the clinical course of VH. The serum level of complement C 3 (β_1C/β_1A-globulin) displayed an overall increase among VH patients relative to controls.

Intestinal bacterial infections showed some differences in the serum level of immunoglobulins in comparison with those in VH: in the former mainly

IgA was increased. A rise in IgG was recorded in salmonellosis and alimentary toxicoinfections. IgM remained within normal limits, or else, it was but slightly reduced. The above data point to the fact that in enteral bacterial infections a substantial effect is exerted mainly on the system IgA- and IgG-antibody production. In accordance with updated concepts, the basic IgA producents (not just secretory IgA, but serum IgA as well) are the immunocytes from the intestinal tract. And yet, despite the fact that the immune system of the gastrointestinal tract possesses its own immunologic characteristic features, there is a likelihood of a physiological unity between immunocytes from the intestinal tract and immunocompetent cells with other localization. This is further corroborated by IgG increase.

Table 3

Serum Immunoglobulin Values in Some Intestinal Infections

Disease	No	Serum immunoglobulins — mg/100 ml		
		IgG	IgM	IgA
Dysentery — bacteriol. confirmed	91	2290±636	95±34	430± 71
Dysentery — clinical	132	2080±328	106±28	384± 69
Salmonelloses Alimentary	40	2416±423	78±24	446± 61
Toxicoinfections	70	2455±261	113±51	452±121
Enterocolitis	89	1830±106	106±60	405± 84
Other enteral infect.	37	1967±121	108±34	303± 77
Controls	300	1582±423	129±61	199± 84

Table 4

Mean Values of Serum Immunoglobulins in Influenza Patients

Sera from influenza patients	No		Serum immunoglobulins — mg/100 ml		
			IgG	IgM	IgA
I investigation	81	$\bar{x}$	1667,41	108,23	137,30
		σ	935,14	62,15	105,11
		m_x	103,90	6,91	11,68
II investigation	68	$\bar{x}$	2497,65	136,61	319,87
		σ	741,14	54,54	137,76
		m_x	89,84	6,61	16,71
Controls	300	$\bar{x}$	1582,11	129,02	199,22
		σ	283,00	61,14	84,01
		m_x	16,33	3,52	4.84

The mean values of immunoglobulins in the repeatedly (twice) studied serum from influenza patients (A_2 Hong Kong 68, B Sofia 59, A_2 Engl. 72 etc.) showed an inrease in Ig A and IgG during the second examination.

Quantitative serum immunoglobulins determination disclosed characteristic changes in chronic hepatic diseases, and in blood diseases, already reported on previously (2, 3), which is an indication that their determination is by no means specific for the various diseases.

The immunochemical methods and, more particularly, radial immunodiffusion actually afford greater possibilities for serum proteins' determination. The adoption of unified standards and control preparations would guarantee the obtaining of comparable results. Regardless of the fact that changes in serum proteins, and chiefly in serum immunoglobulins, are by no means specific, their determinatien after the method desribed has an unquestionable value as a diagnostical, diferential diagnostical and prognostic test for analysis of immune and pathogenic mechanisms of the organism in a variety of diseases.

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ИМУННОХИМИЧЕСКОЕ КОЛИЧЕСТВЕННОЕ ОПРЕДЕЛЕНИЕ НЕКОТОРЫХ ПРОТЕИНОВ СЫВОРОТКИ И ИХ ДИАГНОСТИЧЕСКОЕ ЗНАЧЕНИЕ

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РЕЗЮМЕ

Имуннохимическое количественное определение протеинов сыворотки в настоящее время дает широкую возможность их всестороннего исследования. Наиболее пригодным методом стала радиальная имуннодиффузия в агаре с соответствующими моноспецифическими антисыворотками. В клинике инфекционных болезней — Варна за период с 1973 по 1976 г. исследовано вообщем 1150 больных, из которых с вирусными и бактериальными кишечными инфекциями — 667, хроническими заболеваниями печени — 77 и вирусными капельными инфекциями — 96, заболеваниями крови — 150 и бронхиальной астмой — 50. Определение уровня имунноглобулинов сыворотки (JgG, JgM, JgA, JgE), трансферина, альфа$_2$-макроглобулина, комплемента С 3 (B_1C/B_1A-глобулина), преальбумина, альфа$_1$-антитрипсина, бета$_2$-гликопротеина и др. этим методом имеет несомненную ценность как диагностический тест для анализа имунных и патогенетических механихмов организма при различных заболеваниях.